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ORIGINAL

Determination of Racemic Ketorolac, Ketorolac Enantiomers and Their Metabolites in Human Plasma and Urine by LC–UV, Applied in Clinical Study During and After Pregnancy

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Abstract In order to enhance the sensitivity and to develop a faster direct method for plasma and urine quantification of racemic ketorolac, its metabolites (*p*-hydroxy-ketorolac and ketorolac glucuronides) and ketorolac enantiomers, we developed an extraction procedure based on solid-phase extraction combined with specific and fast chromatographic separation. Extraction and chromatography resulted in cleaner chromatograms without interfering compounds. In both plasma and urine, linearity of the standard curves for racemic ketorolac and *p*-hydroxy-ketorolac was validated in the concentration range 0.025–10 mg L^{-1}, while for ketorolac enantiomers in the concentration range 0.025–5 mg L^{-1}. The lower limit of quantification was two times lower than in earlier described methods. The developed method was suitable for direct quantification of racemic ketorolac, *p*-hydroxy-ketorolac and ketorolac enantiomers in plasma and urine samples in women at delivery and in postpartum, enabling us to document significant intra-individual differences in pharmacokinetics between these physiological states.

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Introduction

In non-pregnant adults, the non-steroidal anti-inflammatory drug ketorolac undergoes elimination through renal (55–60 %) and hepatic metabolic elimination (40–45 %) including both phase I [oxidation via cytochrome P450 (CYP)2C8-9 to *p*-hydroxy-ketorolac, 12–22 %] and phase II [glucuronidation via uridinediphosphateglucuronosyltransferase (UGT)2B7, 21–24 %] [1–3] reactions. When administered intravenously (IV), 91.8 % of the dose (1.7 mg kg^{-1}) is retrieved in 24-h urine collection [2].

Physiological changes in pregnancy and postpartum, including changes in hepatic and renal functions likely affect ketorolac disposition [4, 5]. Despite the limited data on ketorolac pharmacokinetics in those women, it is frequently used as analgesic in this population [6–12], as part of a multimodal post-caesarean analgesia [4, 13–15].

So far, high performance liquid chromatography (HPLC) methods were described to determine racemic ketorolac in human plasma [16–24], racemic ketorolac in human urine [16, 20, 21], *p*-hydroxy-ketorolac in human plasma [18], *p*-hydroxy-ketorolac in human urine [20, 21], ketorolac enantiomers in human plasma [20, 22, 25–29] and ketorolac enantiomers in human urine [20]. Except for solid-phase extraction for racemic ketorolac [19, 23, 24] and for ketorolac enantiomers [22, 29], published sample preparations include mainly liquid–liquid extractions for racemic ketorolac [17, 18, 20, 21] and ketorolac enantiomers [20, 25–28, 30]. Mroszczak et al. [21] described ethyl acetate and hexane extraction

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for racemic ketorolac in plasma. Wu et al. [18] used three-stage liquid–liquid extractions with diethyl ether and hexane for racemic ketorolac and *p*-hydroxy-ketorolac in plasma. Similar extraction was employed by other authors for racemic ketorolac in plasma [17, 20]. For ketorolac enantiomers, indirect HPLC methods based on derivatization and using achiral HPLC were developed first [28–30]. However, only an indirect method [29] based on formation of diastereomeric amides gave satisfactory results while other indirect methods resulted in complete and rapid racemization due to alkaline conditions during derivatization [25]. Nowadays, those assays are typically performed by direct HPLC methods using either chiral HPLC after using methyl *tert*-butyl ether [25], diethyl ether [26], pentan-2-ol [27] or ethyl acetate [20, 30] as extractants or a solid-phase extraction column [29]. The last method prevented racemization, which is mainly the result of usage of strong alkaline and strong acid (pH < 1) reagents. Except for alpha1 acid glycoprotein-based chiral column, a human serum stationary phase was also used [31].

For ketorolac enantiomers in human plasma, HPLC combined with mass spectrometry (MS) has also been described [32].

Because the earlier described methods are both rather time consuming and complicated, we aimed to develop a new HPLC technique to determine racemic ketorolac, ketorolac enantiomers and its metabolites in human plasma and urine. This method was subsequently applied to samples collected at delivery and in postpartum to quantify the impact of pregnancy on ketorolac disposition.

Materials and Methods

Chemicals and Reagents

Racemic ketorolac and *p*-hydroxy-ketorolac were obtained from Hoffmann-La Roche Ltd. (Mannheim, Germany), tolmetin and naproxen from Sigma-Aldrich (Schnelldorf, Germany). Methanol and acetonitrile were HPLC grade and obtained from Bisolve (Valkenswaard, The Netherlands).

Citric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, β-glucuronidase, acetic acid, sodium acetate and 2-propanol were analytical grade and obtained from Merck (Darmstadt, Germany). Millipore ultrapure water was obtained from a simplicity system (Millipore, Belgium), drug-free human urine was obtained from healthy volunteers and drug-free human plasma from the internal plasma bank (Center for Clinical Pharmacology, Leuven, Belgium).

Instrumentation and Chromatographic Conditions

Solid-phase extraction columns (Bond Elut C2 100 mg, 1 mL volume for racemic ketorolac and *p*-hydroxy-ketorolac, Bond Elut C8 100 mg, 1 mL volume for hydrolysed samples, Oasis HLB 30 mg, 1 mL volume for ketorolac enantiomers and a Vac Elut SPS24 vacuum extraction system) were used.

The HPLC system consisted of a Waters 600E HPLC-pump (Milford, MA, USA), a photodiode array detector 996 (Waters) and dual UV detector 2487 (Waters) and an auto-injector WISP 717 plus (Waters). Waters chromatographic data system Empower 2 was used for integration and calculation of the data.

Racemic analytes were separated on a Hypersil BDS C18 5μ column (250 × 4.6 mm i.d.) and the ketorolac enantiomers on a chiral AGP 5μ column (100 × 4.0 mm i.d.). The mobile phase consisted of a mixture of acetonitrile and 15-mM potassium dihydrogen phosphate buffer pH 3.0 (+0.05 % triethylamine) (40/60, *v/v*) for racemic determination and of a mixture of 2-propanol and 50-mM potassium dihydrogen phosphate buffer pH 5.5 (5/95, *v/v* for plasma and 4/96, *v/v* for urine) for enantiomer separation. The mobile phases were pumped with a flow rate of 1 and 0.5 mL min^{-1}, respectively. The PDA detector or UV detector 2487 was set at 313 nm.

Preparation of Standard Solutions and Quality Control Samples

Two individually weighted (1 g L^{-1}) stock solutions of racemic ketorolac and *p*-hydroxy-ketorolac were prepared in methanol and stored at 4 °C for preparation of either the calibration standards or the quality control (QC) samples. In order to obtain calibration standards in the range of 0.025–10 mg L^{-1} for plasma or urine, appropriate methanol/water (50/50, *v/v*) dilutions of racemic ketorolac and *p*-hydroxy-ketorolac stock solutions were performed daily with each new batch of samples and added to aliquots of drug-free human plasma or diluted blank urine. From the second stock solution of racemic ketorolac and *p*-hydroxy-ketorolac low, middle and high QC samples at 0.1, 0.5 and 2.5 mg L^{-1} were prepared in drug-free human plasma or diluted blank urine, then 0.65 mL was aliquoted into polypropylene tubes and stored at −20 °C until analysis.

Internal standard stock solutions (tolmetin for racemic ketorolac and *p*-hydroxy-ketorolac, and naproxen for ketorolac enantiomers) were prepared in methanol at a concentration of 1 g L^{-1}, stored at 4 °C and further diluted to working solution of 10 and 75 g L^{-1}, respectively.

Patient Plasma und Urine Samples

IV bolus ketorolac tromethamine (Taradyl®, Roche, Anderlecht, Belgium) was administered (30-mg ketorolac tromethamine, equal to 20.345 mg of ketorolac) as part of multimodal analgesic approach applied following caesarean delivery in the University Hospitals Leuven, Belgium [5]. To enable

comparison of ketorolac PK at delivery with postpartum state, a group of the same eight women at delivery and 4–5 months postpartum were studied, using the same methodological approach. Five plasma samples per subject were collected at 1, 2, 4, 6 and 8 h after the drug administration. After collection in lithium-heparinised tubes, blood samples were centrifuged for 10 min at 1,350×*g* at 4 °C and plasma separated.

Urine samples were harvested from the first 8-h urine collection after drug administration.

Both plasma and urine were stored at −20 °C until analysis. Before assaying, all samples were allowed to thaw at room temperature for 30 min, vortexed and centrifuged for 5 min at 4 °C at 1,350×*g*.

Extraction Procedures

Extraction Procedure for Free Racemic Ketorolac and p-Hydroxy-Ketorolac in Plasma and Urine

To 500 μL of drug-free human plasma or urine diluted in water (1/20), 50 μL of the standard ketorolac and *p*-hydroxy-ketorolac dilutions (in the range 0.025–10 mg L^{-1}) in methanol/water (50/50, *v/v*) were added. To all samples (drug-free human plasma or urine, patient plasma or urine and QCs), 50 μL of the internal standard tolmetin dilution (10 mg L^{-1}) in methanol/water (50/50, *v/v*) and 500-μL citrate phosphate buffer pH 2.2 were added. In order to equal the volume, additional 50 μL of methanol/water (1:1, *v/v*) was added to the patient samples and QCs.

After vortexing and centrifuging at 1,800×*g* for 5 min, the samples were ready for application to the solid-phase extraction columns (Bond Elut C2 100 mg, 1 mL volume). The solid-phase extraction was performed with a Vac Elut SPS24 vacuum extraction system. After the columns were activated with two times 1 mL methanol and two times 1 mL water, applying slight vacuum to the columns, the prepared standards, controls and samples were passed through the columns at a vacuum pressure of maximum 5 bar. Then 1-mL water was applied, followed by 1-mL methanol/water (10/90, *v/v*) and again vacuum maintained for 5 min. After elution of the columns was performed with two times 0.5 mL methanol, the eluates were evaporated to dryness with an airstream in a water bath at 45 °C for about 20 min and the dried residues were dissolved in 200 μL of the mobile phase. Injection volumes onto the HPLC column varied between 10 and 60 μL.

Extraction Procedure for Racemic Ketorolac Glucuronides in Plasma and Urine

Glucuronide concentrations were obtained by substraction of the free ketorolac or *p*-hydroxy-ketorolac concentrations from the total hydrolysed concentrations.

Hydrolysis of Samples To 200 μL of plasma or urine diluted in water (1/10), 250 μL 0.5 M sodium acetate buffer pH 5 and 50 μL of β-glucuronidase (3,000 IU) in the same sodium acetate buffer were added. Samples were incubated in a water bath at 37 °C for 4 h and then the reaction stopped in ice bath with 500 μL 2 M acetic acid, followed by addition of the 20 μL internal standard tolmetin (10 mg L^{-1}).

Preparation of Standard Curves To 200 μL of plasma or urine diluted in water (1/10), 300 μL 0.5 M sodium acetate buffer pH 5, 50 μL of standard ketorolac and *p*-hydroxy-ketorolac dilutions in methanol/water (50/50, *v/v*) (final concentration range 0.025–10 mg L^{-1}), 20 μL of the internal standard tolmetin (10 mg L^{-1}) in methanol–water (50/50, *v/v*) and 500 μL 2 M acetic acid were added.

After vortexing and centrifuging at 1,800×*g* for 5 min, the samples were ready for application to the solid-phase extraction columns (Bond Elut C8 100 mg, 1 mL volume). The performance of solid-phase extraction and HPLC conditions were as described for the racemic ketorolac and *p*-hydroxy-ketorolac determination.

Extraction Procedure for Free Ketorolac Enantiomers in Plasma and Urine

To 500 μL of plasma or urine diluted in water (1/10), was added 50 μL of standard ketorolac dilutions in methanol/water (50/50, *v/v*) (in the range 0.025–10 mg L^{-1}), 50 μL of the internal standard naproxen dilution (75 mg L^{-1}) in methanol–water (50/50, *v/v*) and as described for the racemic ketorolac and *p*-hydroxy-ketorolac determination 500-μL citrate phosphate buffer pH 2.2. After vortexing and centrifuging at 1,800×*g* for 5 min, the samples were ready for application to the solid-phase extraction columns (Oasis HLB 30 mg, 1 mL volume). The performance of solid-phase extraction and HPLC conditions was as described for the racemic ketorolac and *p*-hydroxy-ketorolac determination.

Extraction Procedure for Glucuronides of Ketorolac Enantiomer in Urine

After several freezing–thawing cycles, the glucuronides of ketorolac enantiomers became instable due to possible hydrolysis, as was proved by the absence of glucuronides after the described hydrolysis procedure. Only free ketorolac enantiomers were found.

Validation Procedures

The validation of the presented method was based on bioanalytical method validation guidelines [33–35].

The linearity of the method was tested by analysing calibration curves with concentrations in fivefold. Calibration

curves were constructed using peak–height ratios of racemic ketorolac, *p*-hydroxy-ketorolac and ketorolac enantiomers, over the internal standard versus the nominal analyte concentration. Linear least-squares regression fit of the calibration curves was performed. Fitting parameters and back-calculated values were obtained by the software program Excel (Microsoft). Acceptance criteria [33] for standard calibration samples were set as follows: 75 % or a minimum of six standards, when back-calculated [including upper limit of quantification (ULOQ)] should fall within ±15 %, except for the lower limit of quantification (LLOQ) when it should be ±20 % of the nominal value. Values falling outside these limits were discarded, provided they do not change the established calculation model. Acceptance criteria for quality control samples were at least 67 % of QCs should be within 15 % of their respective nominal value, 33 % of the QCs (not all *replicates* at the same concentration) may be outside of 15 % of the nominal value.

Intra-assay precision [coefficient of variation (CV %)] and accuracy [percentage deviation from the nominal value (% dev)] were assessed by setting up standard curves using both five standard concentration levels and three QCs (near the lowest standard, near the middle and near the highest standard) in fivefold. Three QCs were analysed daily for inter-assay precision and accuracy determination.

Lower limit of quantification was defined as the lowest concentration of the standard curve with both precision and accuracy within ±20 % of the nominal value.

The specificity of the method was evaluated through the interference with endogenous compounds from drug-free plasma or urine samples. The interference with other co-medicated drugs was checked by analysing patient samples.

The absolute analytical recovery of racemic ketorolac, *p*-hydroxy-ketorolac, tolmetin, *R*-ketorolac, *S*-ketorolac and naproxen can be determined by comparing the analytical results for extracted samples with those for standard solutions. The overall recovery was calculated over the entire range of calibration standards.

The stability was assessed by assaying the QCs in three freeze–thaw cycles and also by leaving it at room temperature for 24 h (short-term temperature stability). Freeze–thaw cycles were performed as follows: four aliquots of the low and high ketorolac and *p*-hydroxy-ketorolac concentrations (0.1 and 2.5 mg L^{-1}) and *R*-ketorolac and *S*-ketorolac concentrations (0.05 and 1.25 mg L^{-1}) were stored at −20 °C for 24 h and thawed unassisted at room temperature; when completely thawed, samples were refrozen for 24 h under the same conditions. The freeze–thaw cycle was repeated two more times and the samples were analysed on the third cycle. Short-term temperature stability was assessed as four aliquots of the low and high ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac concentrations were thawed at room temperature and kept at this temperature for 24 h before being further analysed. The long-term stability of ketorolac, *p*-hydroxy-ketorolac, tolmetin and naproxen stock solutions was evaluated at 4 °C for more than 1 month.

Results and Discussion

We developed a direct HPLC method for determination of racemic ketorolac, the glucuronides and ketorolac enantiomers. Compared to earlier described HPLC methods [19, 29], we used different solid-phase extraction columns and other chromatographic conditions. Three different solid-phase columns were used: Bond Elut C2 for racemic ketorolac, Bond Elut C8 for hydrolysed ketorolac and *p*-hydroxy-ketorolac and Oasis HLB for ketorolac enantiomers, improving selectivity and specificity of our method.

The new methods compare well with previously published methods with regard to suitability and precision, but are more sensitive, faster and easier to perform.

Additional advantages of the present methods are the identical procedures for racemic ketorolac and ketorolac enantiomers determination in both plasma and urine.

A very simple method for racemic ketorolac extraction from plasma was described recently, but the method for urine sample preparation was significantly different from those described in plasma [16]. In contrast, we described simpler, identical extraction procedures for plasma and urine, shortening the time for ketorolac and *p*-hydroxy-ketorolac urine extraction significantly.

Hydrolysis of samples was earlier described only briefly by Kauffman et al. [20] with no validation data.

Representative chromatograms of racemic ketorolac (KTR), *p*-hydroxy-ketorolac (HO-KTR) and tolmetin (TMT) in patient's plasma and urine samples are shown in Fig. 1, and for *R*-ketorolac (R-KTR), *S*-ketorolac (S-KTR) and naproxen (NAP) in patient's plasma and urine samples in Fig. 2. For ketorolac enantiomers, chromatography sequence is based on similar chromatographic conditions as described in the literature. No enantiomers of HO-KTR were ever separated, and there is no information on this topic in the literature.

Calibration Curve and Linearity

As the variance increased in proportion to the concentration, the best fit was obtained using a weighing factor of 1/concentration^2. Calibration curves were prepared for ketorolac and *p*-hydroxy-ketorolac in the range from 0.025 to 10 mg L^{-1}, and for *R*-ketorolac and *S*-ketorolac in the range from 0.025 to 5 mg L^{-1} for both plasma and urine

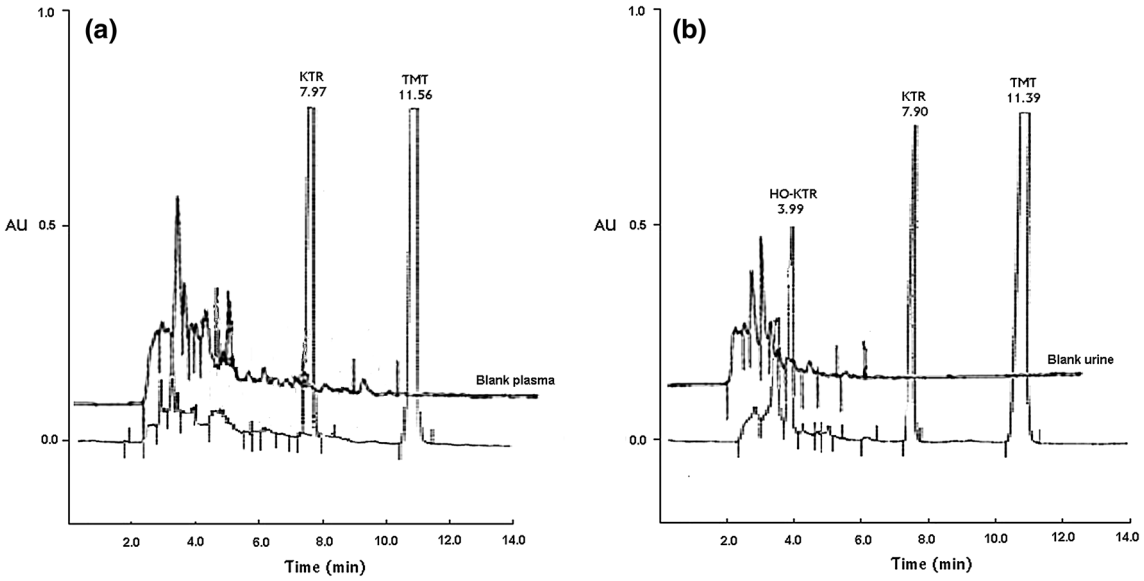

Fig. 1 Chromatogram of **a** blank and patient plasma after drug administration [ketorolac (KTR) plasma concentration 1.73 mg L^{-1}] and internal standard tolmetin (TMT), **b** blank and patient urine sample [KTR urine concentration 5.34 mg L^{-1}, *p*-hydroxy-ketorolac (HO-KTR) urine concentration 1.92 mg L^{-1}] and TMT, extracted as described in "Methodology"

samples. Linearity of calibration curves for plasma and for urine was found with calculated regressions as showed in Table 1. Wider linearity range for racemic ketorolac was observed in our method compared to earlier published methods [16–18, 21]. A wider range of the calibration curve for ketorolac enantiomers (linearity up to 20 mg L^{-1}) was observed earlier [25, 26], but this is of minor clinical relevance in the measurement of low ketorolac enantiomers concentrations (up to 2.01 mg L^{-1}) observed in our clinical plasma samples.

Precision and Accuracy

As a result of the described improvements, we obtained satisfactory intra-assay precision and accuracy as calibration standards and QCs deviated from the nominal value in the acceptable range of CV % and % deviation (all below 15 %, except for *p*-hydroxy-ketorolac and *R*-ketorolac in urine, and for *S*-ketorolac in plasma). Inter-assay precision and accuracy of three QCs for each ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac in plasma and urine are presented in Table 2. Comparable satisfactory precision was shown in earlier studies for racemic ketorolac, e.g. CV <9 % [17, 20], <5.3 % in plasma and <7.2 % in urine [16]. For ketorolac enantiomers, comparable precision was found by direct method [28] and higher CV % than found by other authors e.g. (<10 % [20] and 3–8 %) [22, 30].

Limits of Quantification

The LLOQ for ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac obtained in our study was 0.025 mg L^{-1}, being the lowest concentration of the standard curve with acceptable precision and accuracy (with a coefficient of variation lower than 20 %). The LLOQ for racemic ketorolac in plasma was two times lower when compared to some earlier studies [16, 18, 19], but also higher compared to others in plasma, e.g. 0.005 [20, 36] or 0.003 [17] as well as in urine [16]. This low LLOQ is appropriate for use in pharmacokinetic studies.

The LLOQ for ketorolac enantiomers was comparable to some earlier studies, e.g. 0.02 [22, 29, 30], but also higher compared to others, e.g. 0.01 [37] or 0.005 [20, 25, 26].

The ULOQ for plasma and urine was set as 10 mg L^{-1} for ketorolac and *p*-hydroxy-ketorolac, and as 5 mg L^{-1} for *R*-ketorolac and *S*-ketorolac. Urine samples with concentrations higher than this were re-analysed after appropriate dilution.

Selectivity and Specificity

No interferences with endogenous compounds from pooled human drug-free plasma and drug-free urine were detected using the extraction and chromatographic conditions as described for ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac, tolmetin and naproxen.

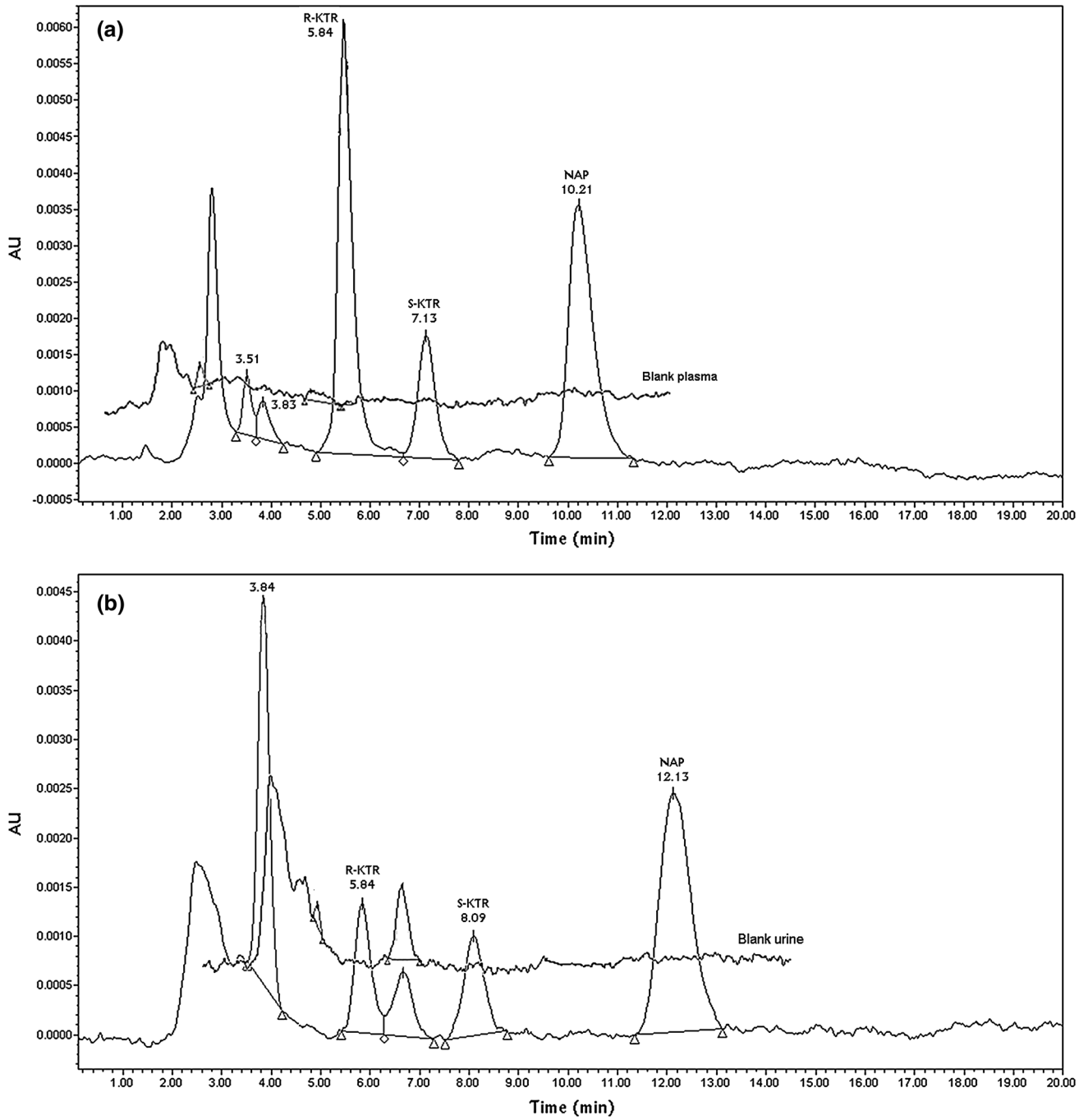

Fig. 2 Chromatogram of **a** blank and patient plasma sample after drug administration [*R*-ketorolac (R-KTR) plasma concentration 1.15 mg L^{-1}, *S*-ketorolac (S-KTR) plasma concentration 0.37 mg L^{-1}] and internal standard naproxen (NAP), **b** blank and patient urine sample collected 8 h after drug administration (R-KTR urine concentration 0.075 mg L^{-1}, S-KTR urine concentration 0.095 mg L^{-1}), and NAP, extracted as described in "Methodology"

Extraction Method and Recovery

Analytical recovery (%) of ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac, *S*-ketorolac, and internal standards tolmetin were almost around 100 %. The relative analytical recovery was determined by comparing peak heights of spiked QCs with peak heights of calibration standards.

Compared to our study where average recovery of racemic ketorolac in plasma was 118.1 % and in urine 126.9 %, in recently published studies it was 99.3 % [16], 98.4 % [18], 80–90 % [17] in plasma and 80.3 % [16] in urine.

Table 1 Linearity of calibration curves: free ketorolac (KTR), *p*-hydroxy-ketorolac (HO-KTR), free *S*-ketorolac (S-KTR) and *R*-ketorolac (R-KTR) enantiomers

	Linearity of calibration curves			
	Linear equation ($y = a + bx$)	*R* (correlation coefficient)	Linear equation ($y = a + bx$)	R (correlation coefficient)
	KTR		HO-KTR	
Plasma free	$-001 + 1.91x$	0.9992	$-0.00 + 3.11x$	0.9966
Urine free	$0.00 + 1.87x$	0.9997	$0.16 + 3.52x$	0.9981
Plasma hydrolysed	$0.01 + 1.71x$	0.9988	$0.38 + 2.79x$	0.9987
Urine hydrolysed	$0.01 + 1.69x$	0.9998	$-0.01 + 2.92x$	0.9991
	R-KTR		S-KTR	
Plasma free	$0.00 + 3.58x$	0.9974	$-0.03 + 2.94x$	0.9965
Urine free	$-0.00 + 4.57x$	0.9974	$0.01 + 3.28x$	0.9964

Table 2 Inter-assay precision and accuracy [expressed as a mean for all plasma and urine samples analysed on free ketorolac (KTR) and *p*-hydroxy-ketorolac (HO-KTR), and plasma and urine samples analysed on free *S*-ketorolac (S-KTR) and *R*-ketorolac (R-KTR) enantiomers] of quality control samples extracted from plasma and urine

Nominal concentration (mg L^{-1})	Inter-assay precision and accuracy					
	Obtained concentration (mg L^{-1})		Precision (CV %)		Accuracy (% dev)	
	Plasma (34 days)	Urine (10 days)	Plasma (34 days)	Urine (10 days)	Plasma (34 days)	Urine (10 days)
KTR						
0.1	0.09	0.09	10.5	6.2	−8.8	−7.5
0.5	0.47	0.46	8.1	4.6	−6.7	−7.9
2.5	2.41	2.32	8.8	5.3	−3.8	−7.2
HO-KTR						
0.1	0.09	0.10	12.9	15.1	−8.1	1.7
0.5	0.47	0.50	10.9	4.4	−5.0	0.0
2.5	2.49	2.56	10.8	8.4	−0.4	2.2
	Plasma (21 days)	Urine (4 days)	Plasma (21 days)	Urine (4 days)	Plasma (21 days)	Urine (4 days)
R-KTR						
0.05	0.05	0.05	13.5	15.2	−8.2	0.2
0.25	0.23	0.22	9.9	5.4	−8.5	−12.6
1.25	1.18	1.15	9.1	5.5	−5.6	−8.3
S-KTR						
0.05	0.05	0.05	15.2	14.9	−3.3	8.0
0.25	0.23	0.22	10.3	12.1	−6.6	−14.0
1.25	1.18	0.15	9.3	5.9	−5.8	−8.0

Average recovery of *R*-ketorolac and *S*-ketorolac in plasma using our method (101 and 96.6 %) was comparable to earlier found recoveries 88.4–110 % and 90.1–110 % found [29], and better compared to other published ones, e.g. 74.33 and 73.15 % [25], 91–94 % and 91–96 % [30].

Stability

Quality control samples were tested at different temperature conditions and stability of ketorolac was satisfactory, with results deviating less than 10 % from nominal values. For *p*-hydroxy-ketorolac, however, deviation higher than 20 % was found. When stored at 4 °C, stock solutions of ketorolac and *p*-hydroxy-ketorolac and ketorolac enantiomers remained stable for more than 3 months, as proven by the reproducibility of the slope of the daily calibration curves (Table 3). Plasma and urine samples were stable for several months when stored at −20 °C, less than 15 % deviation was found re-analysing samples after more than 4 months.

Table 3 Long-term stability of slopes of standard curves/stock solutions: free ketorolac (KTR), *p*-hydroxy-ketorolac (HO-KTR), free *S*-ketorolac (S-KTR) and *R*-ketorolac (R-KTR) enantiomers

	Mean slope		Precision (CV %)		Accuracy (% dev)	
	KTR	HO-KTR	KTR	HO-KTR	KTR	HO-KTR
Plasma free ($n = 25$)	1.582	1.476	6.211	16.711	0.098	0.246
Plasma hydrolysed ($n = 13$)	1.707	2.948	14.291	15.083	0.245	0.445
Urine free ($n = 6$)	1.598	2.443	5.013	8.455	0.081	0.207
Urine hydrolysed ($n = 4$)	1.728	3.048	2.422	3.703	0.041	0.115
	R-KTR	S-KTR	R-KTR	S-KTR	R-KTR	S-KTR
Plasma free ($n = 21$)	3.782	3.012	12.177	11.299	0.462	0.341
Urine free ($n = 3$)	4.290	3.147	2.597	3.000	0.095	0.114

Application of the Methodology in Plasma and Urine Samples of Women at Delivery and in Postpartum

As we proved selectivity, linearity, precision and accuracy, this assay was successfully applied in ketorolac pharmacokinetics studies in the same cohort of women at delivery and 4–5 months postpartum (paired study design) [15].

To illustrate the applicability of the method, plasma and urine concentrations of racemic ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac were quantified in 80 plasma samples and in 16 urine samples collected in eight women at delivery and in 4–5 months postpartum. Racemic ketorolac glucuronide in urine was also quantified by hydrolysis of samples. Data on racemic ketorolac in young women including women at delivery and in postpartum with significant differences in disposition in the same group of women at delivery and 4–5 months postpartum have recently been reported by our group [4, 13–15]. Data on enantiomer-specific disposition are of relevance and in the present study, we would like to focus on pharmacokinetics' differences between ketorolac enantiomers in hereby mentioned group of young women. Representative chromatograms of patient plasma and urine sample are shown in Figs. 1 and 2.

Concentrations in plasma ranged from 0.06 to 2.01 mg L^{-1} and 0.01 to 1.13 mg L^{-1} for *R*-ketorolac and *S*-ketorolac, respectively, and in urine from 1.16 to 23.14 mg L^{-1} and 1.02 to 21.95 mg L^{-1}, respectively.

A preliminary interpretation of time–concentration profiles of racemic ketorolac and ketorolac enantiomers in the same group of women at delivery and in postpartum following single IV administration of ketorolac (30 mg) is shown in Fig. 3. Median clearance and distribution volume estimates for racemic ketorolac were 0.05 vs. 0.04 L h^{-1} kg^{-1} and 0.19 vs. 0.16 L kg^{-1}, for *R*-ketorolac 0.07 vs. 0.05 L h^{-1} kg^{-1} and 0.29 vs. 0.22 L kg^{-1}, and for *S*-ketorolac 0.22 vs. 0.13 L h^{-1} kg^{-1} and 0.59 vs. 0.42 L kg^{-1}. Pharmacokinetic parameters were calculated as reported earlier (racemic ketorolac) [4]. This confirms both racemic ketorolac and the enantiomer-specific elimination to be faster in women at delivery compared

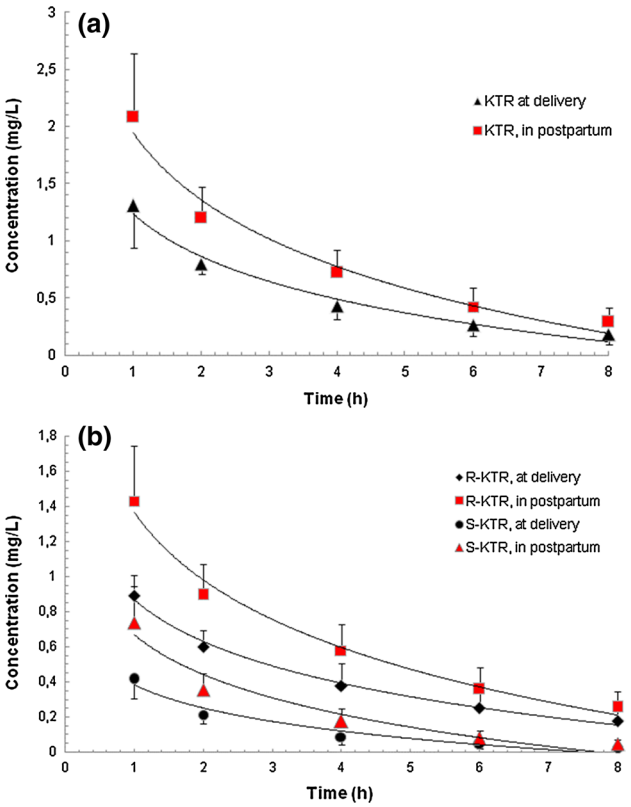

Fig. 3 Mean ± SD time–concentration profiles with a trend line assuming a non-compartmental model of **a** racemic ketorolac and **b** *R*-ketorolac and *S*-ketorolac, in eight women at delivery and 4–5 months postpartum

to the same women 4–5 months postpartum. As proven in non-pregnant adults [22, 38] and children [20, 22, 39], *S*-ketorolac is cleared faster compared to *R*-ketorolac.

In addition, by analysing both plasma and urine samples, we could quantify the portion of racemic ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac, *S*-ketorolac and ketorolac glucuronide excreted in urine and the percentage of dose recovered in 8-h urine collections (Table 4). We documented higher contribution of oxidative metabolic pathways to overall ketorolac clearance at delivery

Table 4 Fractions of free racemic ketorolac (KTR), *p*-hydroxy-ketorolac (HO-KTR), *R*-ketorolac (R-KTR), *S*-ketorolac (S-KTR) and glucuronidated ketorolac (KTR-G) found in the urine following IV ketorolac tromethamine (single bolus dose, 30 mg)

	At delivery ($n = 8$)		Postpartum 4–5 months ($n = 8$)	
	Fractions of dose	Fractions of total drug recovered	Fractions of dose	Fractions of total drug recovered
KTR	0.23 (0.08–0.43)	0.61 (0.32–0.84)	0.21 (0.17–0.29)	0.48 (0.34–0.64)
HO-KTR	0.15 (0.07–0.17)	0.39 (0.16–0.38)	0.12 (0.09–0.16)	0.26 (0.19–0.39)
KTR-G	0	0	0.08 (0.06–0.26)	0.20 (0.14–0.46)
R-KTR	0.10 (0.05–0.25)	0.31 (0.16–0.53)	0.15 (0.12–0.20)	0.34 (0.27–0.40)
S-KTR	0.09 (0.06–0.19)	0.32 (0.21–0.39)	0.13 (0.11–0.19)	0.30 (0.28–0.35)
Dose excreted in urine (8 h)	0.31 (0.25–0.48)		0.45 (0.41–0.56)	

Data are median (min–max value)

compared to 4–5 months postpartum (average 38 vs. 28) [13]. The combined analysis of plasma and urine samples should enable us to search for covariates of the ketorolac clearance capacity in young women including women at delivery and in postpartum as vulnerable populations.

To the best of our knowledge, the most important advantages of our methodology compared to already available methods are the same quantification procedures for plasma and urine, simultaneous analysis of racemic ketorolac and *p*-hydroxy-ketorolac and specific ketorolac enantiomers determination.

Conclusion

An accurate, specific, sensitive and rapid HPLC method for quantification in both human plasma and urine was developed and validated for the determination of ketorolac, *p*-hydroxy-ketorolac, *R*-ketorolac and *S*-ketorolac. The method was subsequently applied in clinical plasma and urine samples, indicating its reliability and suitability for subsequent use in pharmacokinetic studies. Based on plasma and urine analysis we suggest that, when compared to non-pregnant women, overall ketorolac clearance is increased in women at delivery, due to increased *R*-ketorolac as well as *S*-ketorolac clearances. Similar to other populations, *S*-ketorolac is cleared faster compared to *R*-ketorolac.

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